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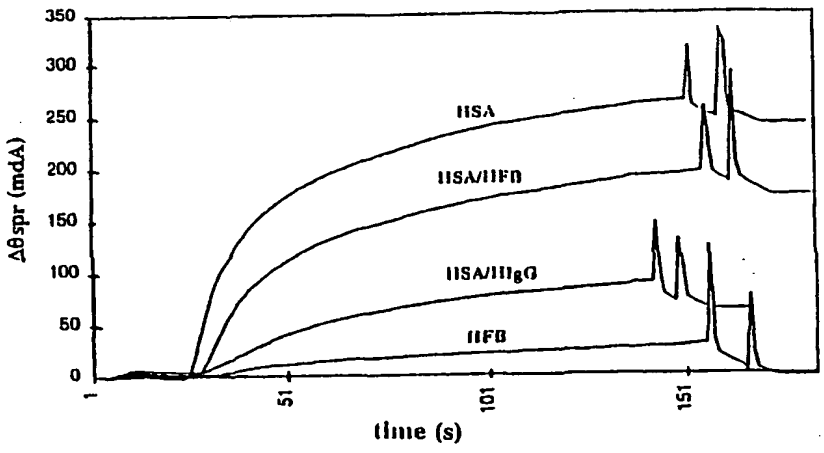
$$CH_2 = \underset{X}{C} - COOCH_2\,Y(CF_2)_n\,CF_2Z \qquad (I)$$



(57) Abstract

Copolymers comprising moieties derived from at least one first fluorinated comonomer having formula (I), wherein X is hydrogen or methyl, Y is a single bond or a radical of the formula CH_2NRSO_2 or $NRSO_2$, R being a C_{1-6} alkyl group, Z is hydrogen or fluorine, and n is 0 to 12 and moieties derived from at least one second non–fluorinated comonomer, the glass transition temperature of the homopolymer of said second comonomer being lower than the glass transition temperature of the homopolymer of said first comonomer, are described. A layer of such a copolymer, optionally further comprising a biologically effective amount of at least one biologically active ingredient, is useful for coating a biomedical device such as a catheter or a stent.

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FLUORINATED COPOLYMERS FOR COATING BIOMEDICAL DEVICES AND A PROCESS FOR THEIR MANUFACTURE.

The present invention relates to a new class of fluorinated copolymers which, due to their good combination of low or negligible cytotoxicity, biocompatibility (especially blood compatibility), infection and encrustation resistance and due to their interaction with tissue cells leading to integration, are useful for biomedical applications. The present invention also relates to a process for manufacturing such fluorinated copolymers. Furthermore, this invention also relates to implantable and invasive biomedical devices such as implants, stents, catheters, staples and the like which are coated with a layer of such a fluorinated copolymer. Another use of such polymers is as a substrate for seeding endothelial cells. Finally, the present invention relates to a method for treating atherosclerosis by making use of a coronary stent coated with a layer of such a fluorinated copolymer, the said layer being optionally loaded with biologically-active ingredients. Thus the invention is in the field of biomaterials, more particularly for stenting of coronary arteries and for other implant therapies.

BACKGROUND OF THE INVENTION

For the treatment of atherosclerosis, it is now common to use a coronary stent made from a metal tubing which is brought to the place of obstruction, i.e. inserted into a vessel, for instance via percutanic transluminal coronary angioplasty and then dilatated, e.g. by a balloon catheter, in order to prevent closure of the said vessel due to a stricture or other external cause of compression. Stents provide a permanent support of the vessel wall and ensure an efficient flow therethrough. However, acute occlusion due to thrombogenecity of the metal and restenosis due to neointimal proliferation of smooth muscle cells tend to impede the success of the presently existing stents. In particular, their thrombotic character induces the need for a simultaneous anti-coagulation therapy which adds to the discomfort of the treatment.

Restenosis of blood vessels occurs after narrowed or occluded arteries are forcibly dilated by balloon catheters, drills, lasers and the like in an angioplastic procedure. Such forcible dilation is required in order to reopen arteries which have been narrowed or occluded by atherosclerosis. However, nearly half of all arteries which have been treated by angioplasty return to their narrowed state through the process of restenosis. Restenosis is caused both by recoil of the vessel wall towards its original dimensions and by neointimal hyperplasia induced by trauma to the vessel wall. Restenosis can therefore significantly reduce the efficacy of angioplasty and as such is a major barrier to the effective treatment of narrowed arteries.

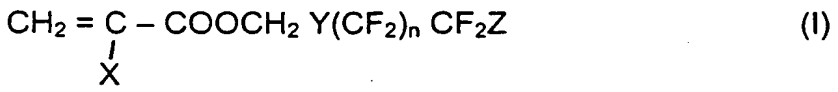
Attempts to reduce or eliminate restenosis have generally focused on the design of biomedical devices, such as stents, within the treated artery. Stents are expected to reduce restenosis by preventing recoil of the treated blood vessel to its original dimensions. Various stents are known in the art, including those which are expandable by balloon catheters, heat expandable or self-expandable. Unfortunately, stents alone cannot prevent restenosis caused by neointimal hyperplasia of the tissues of the vessel wall. In fact, the stent material itself may accelerate such hyperplasia, since it is foreign to the body tissues. Therefore there is a need in the art for coating stents with a material which is not recognized as a foreign body. Further, there is also a need for a stent coating material which can be applied onto the stent body as a flat thin layer, because during stenting, expansion usually leads to a rough surface which will cause more tissue damage, e.g. more restenosis. Solving these problems would open the way to a number of further medical applications since stents are not only used for coronary arteries, but also in the oesophagus for strictures or cancer, the urether for maintaining drainage from the kidneys, or the bile duct for pancreatic cancer or cholangiocarcinoma.

Another requirement of biomedical devices is the resistance to bacterial, viral, fungal and other undesirable infections. This is especially true of some devices which become unworkable after a short period of time and must be replaced from time to time. For example frequent replacement of urinary catheters

may cause excessive discomfort to the patient and prolonged hospitalization. Infections in the case of intravenous catheters used for critical care patients can prove to be life threatening. Therefore there is a need in the art for devices of all kinds exhibiting an improved infection resistance, in particular bacterial resistance.

SUMMARY OF THE INVENTION

The present invention provides a novel class of copolymers comprising moieties derived from at least one first fluorinated comonomer having the formula :



$$CH_2 = \underset{\displaystyle X}{\overset{\displaystyle |}{C}} - COOCH_2\, Y(CF_2)_n\, CF_2Z \qquad (I)$$

wherein

- X is hydrogen or methyl,
- Y is a single bond or a radical of the formula CH_2NRSO_2 , R being a C_{1-6} alkyl group,
- Z is hydrogen or fluorine, and
- n is 0 to 12

and moieties derived from at least one second non-fluorinated comonomer, the glass transition temperature of the homopolymer of said second comonomer being lower than the glass transition temperature of the homopolymer of said first comonomer.

The present invention further provides terpolymers and tetrapolymers additionally comprising moieties derived from at least one hydrophilic, e.g. cationic, third comonomer and/or from at least one non-hydrophilic fourth comonomer copolymerizable with the first comonomer and the second comonomer. In view of their uses for medical applications, the present invention provides such copolymers, terpolymers and tetrapolymers in the form of a layer preferably having a thickness in the range of about 0.1 to 15 µm and optionally further comprising a biologically active ingredient. More specifically, the present invention provides biomedical devices, e.g. implantable and invasive biomedical devices such as implants, catheters (both cardiovascular and urinary), artificial veins and arteries, valves, stents (especially coronary stents comprising a stent body having a metal

surface) and the like coated with at least one such layer and optionally coated with at least one barrier layer. When used in conjunction with a biologically active ingredient for the coating of coronary stents, the copolymers, terpolymers and tetrapolymers of the invention are capable of providing a sustained or controlled release of the biologically active ingredient over an extended period of time. Moreover, the present invention provides use of such a copolymer, terpolymer or tetrapolymer as a substrate for seeding of endothelial cells.

Finally, the present invention further provides a method for treating atherosclerosis by making use of a coronary stent coated with at least one layer of such a copolymer, terpolymer or tetrapolymer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the rate of conversion and the proportions of comonomers, versus time, incorporated into a copolymer of the invention.

Figure 2 shows the sensorgrams of human serum albumin-antibody responses in a surface plasmon resonance study of a copolymer of the invention.

Figure 3 shows antibody binding of various proteins in a surface plasmon resonance study of a copolymer of the invention.

Figure 4 shows radioactive counts for various copolymers and terpolymers of the invention in an *in vitro* bacterial adhesion study via radiolabelling.

Figure 5 shows the detection of adenosine triphosphate for various copolymers and terpolymers of the invention in a bacterial resistance study.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the unexpected discovery that the polymeric combination of a fluorinated monomer and a non-fluorinated monomer able to decrease the glass transition temperature of the polymer sequence derived from the fluorinated monomer results in useful biomaterials. More specifically, it was observed that this polymeric combination, in the form of a copolymer optionally comprising additional hydrophilic and non-hydrophilic comonomers, provides some generally useful biocompatibility properties. The term "biocompatibility" as used herein comprises in particular a low or negligible cytotoxicity, as well as anti-

thrombogenic, anti-inflammatory and anti-immunological properties (i.e. through low interaction with activating blood proteins on cell types and attraction of passivating blood components), bacterial and encrustation resistance. This promising balance of properties may be tailored to specific needs thanks to the wide range of comonomers available within the frame of the invention as well as the various manufacturing possibilities for such polymeric combinations. This may be achieved in particular by designing a copolymer with a specified glass transition temperature (preferably within a range of about -20°C to 20°C) and/or a specified number average molecular weight (preferably within a range of about 25,000 to 200,000) and/or a specified molecular weight polydispersity (preferably within a range of about 1.3 to 5.5).

The copolymers of the present invention first comprise moieties derived from at least one first fluorinated comonomer having the formula :

$$CH_2= \underset{\displaystyle X}{\overset{\displaystyle |}{C}} - COOCH_2\, Y(CF_2)_n\, CF_2Z \qquad (I)$$

wherein

- X is hydrogen or methyl,
- Y is a single bond or a radical of the formula CH_2NRSO_2 or $NRSO_2$, R being a C_{1-6} alkyl group,
- Z is hydrogen or fluorine, and

n is 0 to 12.

Thus, depending on the meaning of X, the comonomer of formula (I) is a fluorinated acrylate or methacrylate optionally (i.e. when Y is not a single bond) bearing a sulfonamido group. As can be seen from the meaning of Z, the comonomer of formula (I) is not necessarily a perfluoro acrylate or methacrylate. The term « C_{1-6} alkyl » as used herein, unless otherwise stated, means a linear or branched alkyl group having from 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, n-hexyl and the like. Non limiting examples of comonomers of formula (I) include trifluoroethyl methacrylate, octafluoropentyl methacrylate, dodecafluoroheptyl

methacrylate, pentadecafluorooctyl methacrylate, heptadecafluorooctyl ethylsulfonamidoethyl acrylate and heptadecafluorooctyl butylsulfonamidoethyl acrylate.

Since the homopolymer of the fluorinated monomer of formula (I) usually (i.e. for a few possible exceptions) usually exhibits a relatively high glass transition temperature (T_g), i.e. a T_g of at least about 25°C, it is generally not entirely suitable for biomedical applications, most of which require some degree of flexibility and elastomeric character which is able to be maintained after sterilization. Therefore it is an essential feature of the present invention that the said first fluorinated monomer of formula (I) be admixed in a polymeric composition together with at least one second non-fluorinated comonomer, the glass transition temperature of the homopolymer of said second non-fluorinated comonomer being lower than the glass transition temperature of the homopolymer of said first fluorinated comonomer. Preferably, the second non-fluorinated comonomer must be copolymerizable in some way with the first fluorinated monomer of formula (I), so that a copolymer comprising moieties from each comonomer species can be obtained. For reasons of biocompatibility, it is preferred that the second non-fluorinated comonomer be an acrylic monomer. The term « acrylic » as used herein, unless otherwise stated, means a monomer bearing either an acrylic or a methacrylic function. In view to achieve a desirable degree of flexibility and elastomeric character for the copolymer of the present invention, it is usually preferred to select the second non-fluorinated comonomer in such a way that the glass transition temperature of the homopolymer of said second comonomer is not above 5°C. A non limiting example of such a non-fluorinated commercial comonomer is 2-ethylhexyl acrylate. Keeping in mind the desirable T_g of the homopolymer, those skilled in the art will readily be able to access additional suitable comonomers, in particular acrylic comonomers.

In view of the biomedical applications for which the copolymers of the present invention have been designed, it is preferred that such copolymers exhibit a glass transition temperature in the range of about -20°C to 20°C. As already mentioned

before, copolymers with a T_g above about 20°C will generally exhibit some kind of brittleness which will make them inappropriate for most biomedical uses, namely for use in implants such as stents and catheters. At the opposite, copolymers with a T_g below about -20°C will generally exhibit some substantial tendency to stickiness which will make them uneasy to handle in manufacturing processes and/or in application of biomedical devices. Tailoring the glass transition temperature of the copolymer of the invention in the range of about -20°C to 20°C is easily available to those skilled in the art and can be achieved by a number of different ways such as, for instance :

- selecting the first fluorinated comonomer within the frame of formula (I),
- selecting the second non-fluorinated comonomer according to the T_g of its homopolymer,
- selecting the respective proportions of said first and second comonomers, namely by making use of Fox's equation as mentioned by L.M.Sperling in « Introduction to physical polymer science » page 357 (John Wiley and Sons, New-York, 1992).

Depending on the choice made for each of the first and second comonomers, the average composition of the copolymers of the present invention may thus be from about 1 to 99 mole %, preferably from about 25 to 75 mole %, of the first fluorinated comonomer and from about 99 to 1 mole %, preferably from about 75 to 25 mole %, of the second non-fluorinated comonomer. As further indicated below, this average composition does not exclude the presence in the copolymer of some fragments or sequences or blocks exclusively constituted from either comonomer moieties.

The detailed structure of the copolymers of the present invention may in some respect play a role in their suitability for biomedical applications and was therefore investigated. This detailed structure, as is well known to those skilled in the art, mostly depends on the process of manufacture of the relevant copolymers. Therefore, the following explanations should be understood as being linked to their obtention via a free radical polymerization process. Within such a process, it was

observed that the copolymers of the present invention most often exhibit a multi-sequence structure with variable proportions of the first comonomer and of the second comonomer in each sequence, i.e. the comonomer proportions are not homogeneous within all macromolecular chains. In some cases, as will be further demonstrated in the following examples, the copolymers of the present invention include at least one block derived from the homopolymer of the first fluorinated comonomer and/or at least one block derived from the homopolymer of the second non-fluorinated comonomer, i.e. they may include a sequence from either or both homopolymers of the building comonomers. This may be derived from the fact that one of the comonomers, usually the fluorinated comonomer of the formula (I), is substantially consumed at the very beginning of the copolymerization reaction (i.e. is incorporated faster in the macromolecular chains) whereas the othe comonomer, usually the non-fluorinated comonomer, is substantially consumed at the very end of the copolymerization reaction. This heterogeneity of at least some of the copolymers of the present invention is also confirmed by the presence of multimodal peaks in their gas permeation chromatograms.

Depending on the type of polymerization process used, it is also possible to obtain the copolymers of the present invention with a branched, grafted, star-shaped or comb-shaped structure.

As already suggested hereinabove, the copolymers of the present invention may conveniently be obtained via a free radical polymerization process. Therefore another object of the present invention is a process for making the said copolymers, comprising providing a mixture comprising :

- at least one first fluorinated comonomer having the formula (I) as stated hereinabove,
- at least one second non-fluorinated comonomer, the glass transition temperature of the homopolymer of said second comonomer being lower than the glass transition temperature of the homopolymer of said first comonomer,

- at least one solvent for the said first fluorinated comonomer and the said second non-fluorinated comonomer, and
- at least one free radical initiator

to a reactor and submitting the said reactor to temperature and pressure conditions under which the said first fluorinated comonomer and the said second non-fluorinated comonomer are able to copolymerize. As a solvent for the said first fluorinated comonomer and the said second non-fluorinated comonomer there can be used a halogenated aliphatic or aromatic hydrocarbon or a mixture thereof. For instance, the said solvent may suitably be selected from 1,1,1-trichloroethane, trichlorotoluene, trifluorotoluene and their mixtures. Preferably, the temperature conditions used in performing the process of the invention include a temperature not exceeding the boiling point of the solvent. If it is desired, the pressure in the reactor may be either raised above or reduced below the normal pressure, although this is in general not necessary. The free radical initiator to be used in the said process may be selected from azoïc compounds such as 2,2'-azobisisobutyronitrile peroxides (e.g. benzoyl peroxide) or conventional alternatives) and redox initiators such as benzoyl peroxide combined with N,N-dimethyltoluidine. Selection of an appropriate free-radical initiator as a function of the selected copolymerization temperature and of the effective amount of such initiator for performing the process of the invention is within the general knowledge of those skilled in the art of polymer synthesis.

An alternative method for preparing the copolymers of the present invention involves anionically copolymerizing a mixture comprising :

- at least one first fluorinated comonomer having the formula (I) as stated hereinabove, and
- at least one second non-fluorinated comonomer, the glass transition temperature of the homopolymer of said second comonomer being lower than the glass transition temperature of the homopolymer of said first comonomer,

at a temperature below about 30°C in the presence of an aprotic solvent, for instance tetrahydrofuran or the like, and further in the presence of at least an anionic polymerization catalyst such as an alcaline metal alkoxide of a tertiary alcohol (e.g. potassium or cesium tertiary butoxide) and/or a crown-ether such as 1,4,7,10,13,16-hexaoxocyclooctane (18-crown-6). As is well known to those skilled in the art, such anionic polymerization can take place at very low temperatures, down to –78°C. Selection of an appropriate anionic polymerization catalyst and of its effective amount for performing such a process is within the knowledge of those skilled in the art of polymer synthesis.

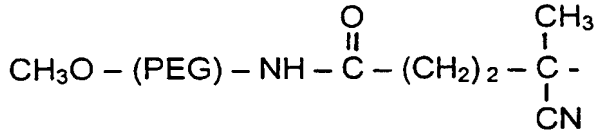
For the purpose of some specific medical applications, it has been found useful to incorporate into the copolymers of the invention, besides the required moieties derived from both the first fluorinated comonomer and the second non-fluorinated comonomer, additional moieties derived from other comonomers, in particular from comonomers which are able to impart some specific physical and /or chemical properties to the resulting copolymers. For instance, in order to impart some hydrophilicity to the copolymers of the invention, the latter may further comprising moieties derived from at least one hydrophilic third comonomer copolymerizable with the first comonomer and the second comonomer. The ability of the third comonomer to copolymerize with the other comonomers is easily determinable by those skilled in the art. For practical reasons, namely for ensuring a good biocompatibility of the resulting terpolymer, it is usually preferred that the the said hydrophilic third comonomer is an acrylic monomer. Many such acrylic monomers may be contemplated for this purpose. For instance, the hydrophilic comonomer may be selected from acrylic acid, methacrylic acid and their alcali or alcaline-earth salts, N,N-dialkylaminoalkyl acrylates and methacrylates (e.g. N,N-dimethylaminoethylmethacrylate), quaternized salts of N,N-dialkylaminoalkyl acrylates and methacrylates (e.g. N,N-dimethylaminoethylmethacrylate benzalkonium chloride useful for its aseptic properties) and polyalkyleneglycol-containing monomers wherein the polyalkyleneglycol sequence (e.g. polyethyleneglycol or polypropyleneglycol) preferably has a molecular weight in

the range of about 400 to 10,000. The latter monomers include, among others, monoacrylates and monomethacrylates as well as monomers derived from polyalkyleneglycol-containing macro-initiators.

Incorporation of such a hydrophilic third comonomer results in the formation of a terpolymer with hydrophilic properties. It may be achieved, when the said hydrophilic third comonomer is not a quaternized N,N-dialkylaminoalkyl acrylate or methacrylate, directly copolymerizing the said hydrophilic third comonomer in a reactor together with the first fluorinated comonomer and the second non-fluorinated comonomer under the conditions stated hereinabove. When the hydrophilic third comonomer is a quaternized N,N-dialkylaminoalkyl acrylate or methacrylate, it is generally preferable to proceed in two steps, by first copolymerizing a N,N-dialkylaminoalkyl acrylate or methacrylate in a reactor together with the first fluorinated comonomer and the second non-fluorinated comonomer under the conditions stated hereinabove (i.e. using a free-radical initiator and a solvent), and then further reacting the terpolymer obtained with at least one halogenated hydrocarbon, preferably a chlorinated aromatic hydrocarbon such as benzyl chloride, in a solvent such as methylene chloride. In this way, hydrocarbon groups such as arylalkyl groups may be covalently linked to the main terpolymer chain via the nitrogen atom of the N,N-dialkylaminoalkyl acrylate or methacrylate.

When the hydrophilic third comonomer is acrylic acid or methacrylic acid or their salts and/or a quaternized N,N-dialkylaminoalkyl acrylate or methacrylate, copolymerization may result in an ionic terpolymer, i.e. a so-called ionomer, obtainable in the form of an aqueous solution following procedures well known to those skilled in the art. When the hydrophilic third comonomer is a polyalkyleneglycol-containing monomer, free-radical copolymerization results in an amphiphilic terpolymer which is especially useful for its protein- and blood platelet-repelling properties. Finally, when the polyalkyleneglycol-containing monomer derives from a polyalkyleneglycol-containing macro-initiator, the latter may be prepared in a two-steps reaction involving, in a first step, reacting an azoïc

compound having terminal carboxylic acid groups, such as 4,4-azobiscyanopentanoic acid, with for instance a N-hydroxyimide in a solvent (such as tetrahydrofuran) and further in the presence of a coupling agent such as cyclohexylcarbodiimide, and in a second step reacting the resulting azoïc compound having terminal imido-ester groups with an α-amino-ω-methoxy-polyalkyleneglycol, the polyalkyleneglycol sequence (e.g. polyethyleneglycol or polypropyleneglycol) preferably having a molecular weight in the range of about 400 to 10,000. Such a polyalkyleneglycol-containing macro-initiator can then be used as an initiator-comonomer in a free-radical copolymerization process together with the first fluorinated and second non-fluorinated comonomers, thus leading to a resulting terpolymer with a terminal moiety having e.g. the formula :



(II)

wherein PEG stands for polyethyleneglycol.

The amount or proportion of hydrophilic third comonomer incorporated into the terpolymers is not critical to the present invention. It usually needs not to be a high proportion in order to impart useful hydrophilic properties to the resulting terpolymer. A proportion of the hydrophilic third comonomer of up to about 40 mole %, preferably from about 1 to 30 mole %, with respect to the combined amounts of the first comonomer and the second comonomer is usually a sufficient amount for the purpose of the present invention. This proportion will also depend upon the nature of the hydrophilic third comonomer ; for instance a proportion of about 1 to 10 mole % should be sufficient when acrylic acid or methacrylic acid constitutes the said hydrophilic third comonomer.

The copolymers of the present invention may further comprise moieties derived from at least one additional non-hydrophilic comonomer (hereinafter the « fourth comonomer ») copolymerizable with the first fluorinated comonomer and the second non-fluorinated comonomer. Various types of comonomers may fall within this category, for instance the said non-hydrophilic fourth comonomer may

be selected for its ability to modify the copolymer surface properties by bearing at least one functional group which is able to react with the copolymer's environment in medical applications, such as the amino groups present in protein. Again for practical reasons of biocompatibility, the said non-hydrophilic fourth comonomer should preferably be an acrylic monomer. A non limiting example of such a comonomer is succinimidyl methacrylate. Incorporation of such a non-hydrophilic fourth comonomer results in the formation of a ter- or tetrapolymer with additional specific physical or chemical properties, including a suitable reactivity with amino groups. For instance, as demonstrated in the following examples, the succinimidyl methacrylate moieties are able to react with an α-amino-ω-methoxy polyethyleneglycol in order to introduce polyethyleneglycol methacrylamido groups into the polymeric chain. The amount or proportion of the non-hydrophilic fourth comonomer is not critical to the present invention. It usually needs not to be a high proportion in order to impart additional useful properties to the resulting ter- or tetrapolymer. A proportion of the non-hydrophilic fourth comonomer of up to about 15 mole %, preferably from about 1 to 10 mole %, with respect to the combined amounts of the first and second comonomers – and optionally third comonomer - is usually a sufficient amount for the purpose of the present invention.

Although this is not a limiting factor of the present invention, it was observed that the copolymers and terpolymers of this invention most often exhibit a number average molecular weight in the range of about 25,000 to 200,000, more preferably about 30,000 to 130,000 and/or a molecular weight polydispersity in the range of about 1.3 to 5.5, more preferably about 1.5 to 3.5, i.e. a relatively narrow distribution of molecular weights.

In view of biomedical applications, it is generally desirable that the copolymers of this invention may be sterilized. This may conveniently be effected by means of irradiation, for instance via electron-beam or X-rays. As is well known to those skilled in the art, the choice between electron-beam and X-ray technologies will mostly depend on the size and thickness of the material to be irradiated. For the complete destruction of all living micro-organisms usually

involved with infection of biomedical equipment, irradiation doses within a range of about 0.5 to 100 kGy, preferably 10 to 50 kGy are recommended.

In view of the main biomedical applications of this invention, it is generally desirable, and this has proven to be easily feasible, that the copolymers of the present invention may be processed in the form of flat thin layers,. The term « thin layer » as used herein, unless otherwise stated, means a layer that can obtained by either of the conventional dip coating and spray coating techniques. This usually corresponds to a thickness in the range of about 0.1 to 15 µm. As already explained hereinabove, the need for a flat layer is especially crucial for the coating of stents. Hence stents coated with a copolymer layer according to the invention will help reducing restenosis and therefore improve the success of angioplasty.

For many biomedical applications of the copolymers of the present invention, it is generally useful that such copolymer layers may further comprise a biologically effective amount of at least one biologically active ingredient such as a therapeutic, diagnostic or prophylactic agent. The said therapeutic agent can be selected for its specific properties such as for instance its anti-thrombotic, anti-inflammatory, anti-proliferative or anti-microbial efficiency. The latter include for instance anti-microbial agents such as broad spectrum antibiotics for combating clinical and sub-clinical infections. Other therapeutic agents which can be considered for incorporation into the copolymer layers of this invention may be naturally occurring or synthetic organic or inorganic compounds, including proteins and peptides (produced either by isolation from natural sources or recombinantly), hormones, carbohydrates, antineoplastic agents or anti-proliferative agents, anti-inflammatory agents, antiangiogenic agents, vasoactive agents, anticoagulants, immunomodulators, cytotoxic agents, antiviral agents, antibodies, neurotransmitters, oligonucleotides, lipids, plasmids, DNA and the like. Therapeutically active proteins which can additionally be present in the formulations of this invention include fibroblast growth factors, epidermal growth factors, platelet-derived growth factors, macrophage-derived growth factors such as granulocyte macrophage colony stimulating factors, ciliary neurotrophic factors,

cystic fibrosis regulator genes, tissue plasminogen activator, B cell stimulating factors, cartilage induction factor, differentiating factors, growth hormone releasing factors, human growth hormone, hepatocyte growth factors, immunoglobulins, insulin-like growth factors, interleukins, cytokines, interferons, tumor necrosis factors, nerve growth factors, endothelial growth factors, non-steroidal anti-inflammatory drugs, osteogenic factor extract, T cell growth factors, tumor growth inhibitors, enzymes and the like, as well as fragments thereof. Diagnostic agents which can be present in the copolymer layers of this invention include conventional imaging agents (for instance as used in tomography, fluoroscopy, magnetic resonance imaging and the like) such as transition metal chelates. Such agents should be incorporated into the layers of the invention in an effective amount for performing the relevant diagnostic. As is well known to those skilled in the art of pharmacology, the at least one biologically active ingredient may be formulated together with additional pharmaceutically acceptable excipients and carriers, as well as together with a biological delivery system able to perform a specific pharmacological function, such as for instance a composition for providing a controlled or sustained release of the said biologically active ingredient into the body of a mammal, in particular of a human patient in need of a specific therapy.

As will readily be understood by those skilled in the art of such biomedical applications, the nature and amount of the at least one biologically active ingredient incorporated in the copolymer layers of the present invention depends on the specific biological action to be performed when implanting a device coated with such a layer into the body of a patient in need of a specific therapy.

Another embodiment of the present invention consists of a biomedical device, for instance an implantable or invasive biomedical device, coated with at least one layer comprising a copolymer as hereinabove described (i.e. including ter- and tetrapolymers possibly incorporating a third and/or fourth comonomer) and possibly further comprising a biologically effective amount of at least one biologically active ingredient. The form, shape and size of the biomedical device is not critical for the present invention. Non limiting examples of implantable and

invasive biomedical devices which fall within the framework of this invention include, for instance, catheters, stents, staples, threads, needles, pacemakers, valves, artificial veins and arteries, electronic pumps and sensors, tubings, prostheses, i.e. implants and materials of all kinds designed for use in the body of a mammal, and more specifically for the human body. Such implantable and invasive biomedical devices are well known to those skilled in the art of surgery making use of implants. If necessary for some end uses, they may additionally be coated with at least one barrier layer. Preferably, the biomedical devices of this invention have a bio-inert surface, for instance a hydrophobic surface with low surface energy and possibly with amphiphilic heterogeneous microdomains. The biomedical device of this invention may also usefully exhibit antagonist properties namely against protein activation, cell activation (e.g. anti-inflammatory), bacteria, and/or anti-thrombotic properties, encrustation repellency and the like.

In particular, the biomedical device of this invention may be a stent, for example a coronary stent, comprising an expandable stent body having a plastic or metal surface and wherein the copolymer layer is coated onto at least a part of the said surface, for instance onto at least one side thereof. Such a stent is particularly useful for the treatment of atherosclerosis, consequently the present invention also provides a method for such treatment by making use of, i.e. by implanting into the body of a patient in need of such treatment, a coronary stent coated with a copolymer layer (such as disclosed hereinbefore) on at least a part of its surface. Moreover, another embodiment of the present invention consists of a process for making a coated stent, comprising coating a stent body having a non-biocompatible surface, for instance a metal or plastic surface, with at least one polymer, the said polymer comprising a copolymer as hereinabove described (i.e. including ter- and tetrapolymers possibly incorporating a third and/or fourth comonomer) and possibly further comprising a biologically effective amount of at least one biologically active ingredient. Coating may be effected by any of the conventional dip coating or spray coating techniques and is preferably effected in a manner such as to provide a thin copolymer layer such as hereinabove described.

As is readily understandable by those skilled in the art, the use of the copolymer layer of this invention in biomedical applications, especially in implantable and invasive biomedical devices, is among others based on a satisfactory adhesion to the copolymer of those proteins which are abundantly present in plasma and which fulfill transport and passivating functions in the last step of the coagulation cascade in immune reactions such as albumin, fibrinogen and immunoglobulin G. Adherence of such proteins is believed to facilitate endothelial cell adherence. The formation of a thin natural endothelial layer, possibly obtained via tissue engineering techniques such as cell seeding, will substantially prevent the surface, for instance the metal surface, of the said implantable or invasive biomedical device to be recognized as a foreign body by the cells of the patient in which the device is implanted. Tissue reactions such as inflammation, severe immunological reactions, thrombus formation, accute occlusion or restenosis are thereby minimized or suppressed. A steady or controlled release of restenosis-suppressing agents over a period of about 10 to 30 days would also allow to inhibit the start of the restenosis process. Protein adhesion was found to be especially satisfactory for those terpolymers of the invention which include moieties derived from a cationic third comonomer, such as a quaternized N,N-dialkylaminoalkyl acrylate or methacrylate or a (meth)acrylic acid or acid salt.

The present invention will now be explained in further details by reference to the following exampleswhich are provided for illustrative purposes only and without any limiting intention.

EXAMPLE 1 – preparation of octafluoropentylmethacrylate

100 g (0.43 mole) octafluoropentanol from Acros Chemical was dissolved in 100 ml methylene chloride. After addition of 60 ml triethylamine (0.43 mole), the solution was cooled down to 0°C. Next, 65 ml methacrylic acid anhydride (0.43 mole) was added dropwise. After 4 hours reaction at 40°C, the reaction mixture was further stirred overnight at room temperature. In order to neutralize traces of unreacted methacrylic acid anhydride, the reaction mixture was reacted during 20

minutes with 5.2 ml (0.086 mole) aminoethanol and then diluted with 100 ml CH_2Cl_2 and 100 ml water and additionally stirred for 1 hour. Extraction of the organic phase was then effected respectively by means of a 3M HCl solution (3 times), a saturated NaCl solution (3 times) and a $NaHCO_3$ solution (3 times). The resulting CH_2Cl_2 phase was dried with $MgSO_4$ and evaporated, leaving, after vacuum distillation, octafluoropentylmethacrylate with a yield of 83 %.

Monomer characterization was effected by means of ^{1}H-NMR in deuterated acetone and provided the following spectrum: $\delta(CH_3)$ 1.92 ppm (s), $\delta(OCH_2)$ 4.82 ppm (t), $\delta(CH_2=C)$ 5.78 ppm (s) and 6.17 ppm (s), $\delta(CH_2F)$ 6.78 ppm (tt).

EXAMPLE 2 - preparation of succinimidyl methacrylate

In a two-neck reactor vessel, 4.3 g of N-hydroxysuccinimide from Acros Chemical (0.043 mole) and 30 ml dichloromethane (CH_2Cl_2) were added. Only after addition of 4.4 g (0.043 mole) of triethylamine, a clear mixture was obtained. After cooling down to 0°C, 3 g (0.029 mole) methacrylic acid chloride was added. After 5 hours reaction at 0°C, the reaction mixture was poured into 40 ml of a saturated $NaHCO_3$ solution. After 2 times extraction with 40 ml of a saturated $NaHCO_3$ solution, and 2 times extraction with 40 ml water, the organic phase was dried with $MgSO_4$, filtered and evaporated. The solid succinimidyl methacrylate resulting monomer was vacuum dried and characterized by ^{1}H-NMR in $CDCl_3$, providing the following spectrum: $\delta(CH_3)$ 2.05 ppm (s), $\delta(CH_2)$ 2.85 ppm (s), $\delta(CH_2=C)$ 5.87 ppm (s) and 6.42 ppm (s).

EXAMPLE 3 – preparation of a poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate)

10g octafluoropentylmethacrylate prepared according to example 1 (0.333 mole), 2.05 g of 2-ethylhexyl acrylate (0.111 mole) were brought to a polymerization tube and dissolved in 50 ml CH_3CCl_3 (i.e. 25% weight/volume) together with 0.23 mmole 2,2'-azobisisobutyronitrile. The polymerization mixture was frozen in liquid nitrogen and degassed under vacuum. This was repeated trice, after which the polymerization tube was sealed under vacuum. After 24 hours heating at 65°C, the resulting copolymer obtained with a yield of 93% was

precipitated twice in pentane and vacuum dried. Follow-up of the amounts of incorporated monomer and unreacted monomer via gas chromatography (as further detailed in example 9) and via ^{1}H-NMR performed on a Brucker WH 360 MHz apparatus (namely the peak areas for the OCH_2 groups of the fluorinated comonomer at δ = 4.55 ppm and of the non-fluorinated comonomer at δ = 3.9 ppm) shows that the fluorinated comonomer was incorporated faster, i.e. in the starting phase preferentially the fluorinated comonomer is incorporated, next a random copolymer is formed and finally the remaining non-fluorinated comonomer reacts. This heterogeneity explains the existence of multimodal peaks for the copolymer in its gel permeation chromatogram.

The glass transition temperature of this copolymer (measured by means of a thermal analysis differential scanning calorimeter DSC 2920, at heating and cooling speeds of 10°C/min.) is 14°C. Its average number molecular weight (determined by gel permeation chromatography while using a Styragel mixed B column, 10µ, 2 x 30 cm from Polymer Laboratories on N-methylpyrrolidone and using polystyrene as a standard) is 89,000 and its molecular weight polydispersity M_w/M_n is 1.7.

EXAMPLE 4 – preparation of a poly(pentadecafluorooctylmethacrylate-co-2-ethylhexylacrylate)

The procedure of example 3 is repeated, except that 0.65 mole of pentadecafluorooctylmethacrylate, either prepared in accordance with a procedure similar to example 1 or originating from Polyscience (Germany), is copolymerized with 0.35 mole 2-ethylhexyl acrylate while using trichlorotrifluoroethane or trifluorotoluene as the reaction solvent. The copolymer is obtained with a yield of 87%. The glass transition temperature (measured as in example 3) of this copolymer is 13°C. Its average number molecular weight (determined as in eample 3) is 123,000 and its molecular weight polydispersity M_w/M_n is 1.8.

EXAMPLE 5 – preparation of a poly(trifluoroethylmethacrylate-co-2-ethylhexylacrylate)

The procedure of example 3 is repeated, except that 0.5 mole of trifluoroethylmethacrylate, either prepared in accordance with a procedure similar to example 1 or originating from Polyscience (Germany), is copolymerized with 0.5 mole 2-ethylhexyl acrylate. The copolymer was obtained with a yield of 95%. The glass transition temperature of this copolymer (measured as in example 3) is 14°C. Its average number molecular weight (determined as in example 3) is 40,000 and its molecular weight polydispersity M_w/M_n is 3.0.

EXAMPLE 6 – preparation of a poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate-co-dimethylaminoethylmethacrylate)

Terpolymerization is effected according to a procedure similar to that of example 3, except that 0.37 g dimethylaminoethylmethacrylate is added into the polymerization tube. A yield of 80% of terpolymer was obtained. Characterization of the said terpolymer was effected by ^{1}H-NMR in $CDCl_3$ providing the following spectrum (for the identification of each signal, dimethylaminoethylmethacrylate was abbreviated as DM, 2-ethylhexylacrylate as EHA and octafluoropentylmethacrylate as FMP): $\delta(NCH_{3\text{-}DM})$ 2.25 ppm, $\delta(NCH_{2\text{-}DM})$ 2.55 ppm, $\delta(OCH_{2\text{-}DM})$ 4.05 ppm, $\delta(OCH_{2\text{-}EHA})$ 3.85 ppm, $\delta(OCH_{2\text{-}FMP})$ 4.55 ppm, $\delta(CF_2H)$ 5.85-6.35 ppm (t).

EXAMPLE 7 – preparation of a quaternized poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate-co-dimethylaminoethylmethacrylate)

To 12.5g of the terpolymer obtained in example 6 (comprising 0.0238 mole dimethylaminoethylmethacrylate) in solution in CH_2Cl_2 (30% (weight/volume) was added 3.02g of benzylchloride (0.238 mole). After 48 refluxes, a quaternized terpolymer was obtained with a yield of 91% via precipitation in pentane and was characterized by ^{1}H-NMR in $CDCl_3$, providing the following spectrum (monomer abbreviations are as in example 5): $\delta(NCH_{3\text{-}DM})$ 3.45 ppm, $\delta(NCH_{2\text{-}DM} + OCH_{2\text{-}EHA})$ 3.65-4.00 ppm, $\delta(OCH_{2\text{-}DM} + OCH_{2\text{-}FMP})$ 4.40ppm, $\delta(NCH_{2\text{-}DM}$, α of aromatic ring) 5.13 ppm, $\delta(CF_2H)$ 5.85-6.35 ppm (t), δ(aromatic H) 7.3-7.8 ppm.

EXAMPLE 8 – preparation of a poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate-co-succinimidyl methacrylate)

A procedure similar to that of example 3 is used, except that 0.7125 mole of octafluoropenttylmethacrylate prepared according to example 1 is copolymerized with 0.2375 mole of 2-ethylhexylacrylate and 0.05 mole of succinimidyl methacrylate prepared according to example 2, while using methylene chloride as the reaction solvent. The copolymer was obtained with a yield of 90%.

EXAMPLE 9 - preparation of a poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate-co-polyethyleneglycolmethacrylamide)

1 g of the terpolymer prepared in example 8 was dissolved in 2 ml of CH_2Cl_2, then 0.22 g of an α-amino-ω-methoxy polyethyleneglycol (also dissolved in CH_2Cl_2) obtained from Fluka AG and having a molecular weight of 750 (i.e. 17 ethyleneglycol units) was added thereto. The mixture was refluxed during 48 hours at 60°C. The resulting viscous solution was diluted with an additional 4 ml of CH_2Cl_2 and subsequently extracted with a saturated solution of $NaHCO_3$ (twice 10 ml) and then with water (twice 10 ml). The dichloromethane phase was dried by means of $MgSO_4$ and evaporated and the resulting terpolymer, bearing polyethyleneglycol methacrylamido groups, was further vacuum dried and characterized by ^{1}H-NMR in $CDCl_3$ providing the following spectrum (monomer abbreviations are as in example 6, with PEG designating polyethyleneglycol): $\delta(OCH_{3\text{-}EHA})$ 3.38 ppm (s), $\delta(OCH_{2\text{-}PEG})$ 3.65 ppm, $\delta(OCH_{2\text{-}EHA})$ 3.90 ppm, $\delta(OCH_{2\text{-}FMP})$ 4.45 ppm, $\delta(CF_2H)$ 5.85-6.35 ppm (t). Infrared spectroscopy (by means of Perkin-Elmer 1600 FTIR) additionally shows the appearance of polyether groups at 1272 and 1259 cm^{-1} and therefor confirms the resulting structure. Similar syntheses were performed with α-amino-ω-methoxy polyethyleneglycols having molecular weights from 400 to 4,000 and the resulting terpolymers were used for protein adhesion tests via surface plasmon resonance in example 14 and for sterilization tests in example 17 below.

EXAMPLE 10 – Determination of the degree of conversion in the radical copolymerisation of octafluoropentylmethacrylate and 2-ethylhexylacrylate by means of gas chromatography.

0.01mole of a monomer mixture of octafluoropentylmethacrylate and 2-ethylhexylacrylate was weighed in a 10 ml flask. 8.2 mg 2,2'-azobisisobutyronitrile (free radical initiator) and 0.67 g n-decane (internal standard) were added. The mixture was further diluted with $CHCl_3$ to a total volume of 10 ml and brought to a two-neck flask, which was sealed with a septum and a valve. Degassing was done by bringing the reaction mixture several times briefly under vacuum. During copolymerization, effected at 65°C like in example 3, an inert nitrogen atmosphere was maintained. During 28 hours, at regular time intervals, a 50 µl sample was taken and diluted chloroform to 300 µl. 0.5 µl was injected on a polydiphenyldimethylsiloxane column (RSL-200 bonded FSOT, 30 m x 0.25 mm, split injection). After having maintained during 4 minutes a temperature of 35°C, the oven was heated to 250°C at a rate of 10°C/min. The retention times for CH_3CCl_3, octafluoropentylmethacrylate (FMP), n-decane and 2-ethylhexylacrylate (EHA) were respectively 1'4, 8'0, 8'6 and 12'7. A calibration curve for concentrations of FMP and EHA between 5 and 20 ng/ml was set in advance. The peak area of each comonomer, divided by that of n-decane, at initial time (t=0) was set to 100% of the corresponding monomer. From the percentages of comonomers present in the reaction mixture, the percentage of comonomer incorporated in the copolymer structure and the conversion rate of the reaction were deduced. Curve fitting was performed using the program "Graphpath Prism" (non-linear regression). Figure 1 shows results of this experiment for the copolymer obtained from a mixture of 50 mole% FMP and 50 mole% EHA. As an additional information, it may be noted that the glass transition temperature of this copolymer is 2°C, its average number molecular weight (determined by gel permeation chromatography) is 57,000 and its molecular weight polydispersity M_w/M_n is 1.8.

EXAMPLE 11 – determination of copolymer compositions by ^{1}H-NMR.

The composition of the copolymers of examples 3-5 and 10 was determined, after recording their ^{1}H-NMR spectra in a deuterated acetone/chloroform (1/1) mixture, on the basis of the OCH_2 peak integrations for

the fluorinated comonomer (e.g. δ = 4.45 ppm for FMP) and for 2-ethylhexylacrylate (δ = 3.90 ppm). Results are as follows :

Copolymer of example 3 contains 81 mole% octafluoropentylmethacrylate and 19 mole% 2-ethylhexylacrylate. Copolymer of example 4 contains 67 mole% pentadecafluorooctylmethacrylate and 33 mole% 2-ethylhexylacrylate. Copolymer of example 5 contains 51 mole% trifluoroethylmethacrylate and 49 mole% 2-ethylhexylacrylate. Copolymer of example 10 contains 63 mole% octafluoropentylmethacrylate and 37 mole% 2-ethylhexylacrylate.

EXAMPLE 12 – preparation of polyethyleneglycol-containing macro-initiators.

A polyethyleneglycol macro-initiator was prepared following a procedure similar to that described by A.Ueda et al. in *J.Polym.Sci., Part A, Polym.Chem.*(1986) 24:405. 1 g of 4,4-azo-bis (4-cyanopentanoic acid) and 0.83g N-hydroxysuccinimide were dissolved in 10 ml tetrahydrofurane. 1.48 g dicyclohexylcarbodiimide in 5 ml tetrahydrofurane was slowly added to the reaction medium at 0°C. After 24 hours stirring, dicyclohexylurea formed by the reaction was removed by filtering. The solvent was evaporated and the azoïc compound with terminal ester groups produced was dissolved in ethyleneglycol dimethylether. Next 7.12 mmole of an α-amino-ω-methoxy-polyethyleneglycol with a molecular weight of 750 to 5,000 from Fluka AG (dried via azeotropic distillation with toluene) in 20 ml dry CH_2Cl_2 was added to the azoïc compound with terminal ester groups. After 24 hours reaction at room temperature, the polyethyleneglycol-containing macro-initiator formed was precipitated in pentane, vacuum dried and further characterized by infrared spectroscopy (linking amide at 1716 cm^{-1}, polyethyleneglycol ether at 1113 cm^{-1}, methylene at 2885 cm^{-1}) and ^{1}H-NMR in $CDCl_3$: δ(CH_3) 1.7 ppm, δ(OCH_2) 2.4 ppm and 2.5 ppm, δ(OCH_3) 3.4 ppm, δ(OCH_2) 3.65 ppm.

EXAMPLE 13 – preparation of poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate-co-polyethyleneglycol derived monomer).

A polyethyleneglycol macro-initiator according to example 12 was brought, in a proportion (from 0.5 to 2% by mole of the total monomers) specified in the following table 1, to a polymerization tube, then 1 g octafluoropentylmethacrylate (3.33 mmole) and 0.205 g of 2-ethylhexylacrylate (1.11 mmole) were added and toluene was used as the polymerization solvent. The polymerization mixture was frozen in liquid nitrogen and degassed under vacuum. This was repeated trice, after which the polymerization tube was sealed. After 24 hours of polymerization in a thermostatic bath at 65 °C, the resulting terpolymer was precipitated twice in pentane and vacuum dried. A thin film was prepared from the said terpolymer via solvent casting from a CH_2Cl_2/acetone (80/20) solution and was extracted during 24 hours at 55 °C with different portions of water, the water fraction was freeze dried and the isolated product identified by means of ^{1}H-NMR-spectroscopy in $CDCl_3$ as follows: $\delta(OCH_{3\text{-PEG}})$ 3.4 ppm (s), $\delta(OCH_{2\text{-PEG}})$ 3.6 ppm, $\delta(OCH_{2\text{-EHA}})$ 4.0 ppm, $\delta(OCH_{2\text{-FMP}})$ 4.7 ppm, $\delta(CF_2H)$ 5.3-6.3 ppm.

Using this procedure, terpolymers with variable proportions of polyethyleneglycol sequences having various molecular weights from 750 to 5,000 were prepared and used for protein adhesion tests via surface plasmon resonance in example 15 and for sterilization tests in example 18 below. Their physico-chemical properties are shown in table 1 below.

Table 1

Terpolymer reference	M_n (PEG)	% mole PEG	T_g (°C)	Cristallinity (%)
13 A	750	0.5	- 7	0.1
13 B	750	2.0	- 20	1.1
13 C	2,000	1.0	- 10	1.2
13 D	5,000	0.5	3	5.1
13 E	5,000	1.0	10	11.7

Cristallinity was determined by X-ray measurements.

EXAMPLE 14 – preparation of a poly(octafluoropentylmethacrylate-co-2-ethylhexylacrylate-co-methacrylic acid).

10 g octafluoropentylmethacrylate, 2 g 2-ethylhexylmethacrylate and 0.2 g methacrylic acid were dissolved in chloroform, then 0.5 mole % 2,2'-azobisisobutyronitrile was added. The reaction vessel was degassed three times and then sealed. Polymerization was allowed to proceed at 65°C for 24 hours. The resulting terpolymer, obtained with a yield of 87 %, was precipitated in pentane, filtered and dried.

EXAMPLE 15 – protein adhesion to copolymer surfaces measured through surface plasmon resonance.

Surface plasmon resonance (SPR) is an optical technique via which adsorption to a material can be followed *in situ*. Laser light incides at the underside of a prism optically coupled to a microscopy glass covered by a thin gold or silver layer. Total internal reflection takes place if light goes to a medium with smaller refractive index and if the angle of incidence is bigger than the critical angle. Under a well defined angle, the resonance angle θ_{spr}, resonance takes place between the extinguishing wave of the photon and the free oscillating surface electrons from the metal layer (plasmons). Hereby, a minimal intensity of the reflected light beam arises and is registered by a photodiode array detector. The angle under which this occurs depends on the refractive index and on the thickness of the absorbed layer. A sensogram is obtained by recording the shift in resonance angle ($\Delta\theta_{spr}$) versus time, the slope of the curve being a measure for the amount of adhered material.

Protein adsorption takes place immediately after introduction of a foreign body into the body of a mammal and this initially adsorbed protein layer further influences physiological and cellular processes such as blood coagulation, fibrinolysis, thrombogenesis, leukocyte activation, immunological and inflammatory responses. The driving interaction forces, such as H-bridges, electrostatic and hydrophobic interactions, for protein adhesion are non-covalent. Protein adsorption is a complex, competitive process whereby some proteins can desorb again and be replaced by other proteins. Many factors, such as affinity of the protein for the

surface, contact time, concentration and flow speed of the protein play a role in this substitution process. When a protein has a high affinity for the surface, it will undergo conformational changes so that multiple bonds with the surface are possible. The longer a protein remains attached, the more it will spread and the stronger the bond is. An irreversible protein monolayer is then formed. Affinity itself is determined in part by the protein, in part by the material surface properties.

The Vroman effect is based on the substitution of proteins and suggests that proteins present in high concentrations bind first and are later replaced by other proteins present in smaller amounts, although with high affinity for the surface. Plasma proteins such as albumin, immunoglobulin G, fibrinogen, fibronectin, Hageman factor, high molecular weight kininogen and high density lipoprotein (HDL) will adhere sequentially.

Material surfaces entail a differential humoral and cellular activity, depending on the protein composition and conformation in the adsorbed layer. A change in conformation can entail activation but also inhibition of the biological function of the protein. Generally speaking, extremely hydrophilic materials impede protein adsorption whereas extremely hydrophobic materials adhere a strongly attached monolayer. Micro-heterogeneous materials usually adsorb proteins in an ordered way, whereby changes in protein structure and cell activation are avoided.

In the following experiment, it will be shown to which extent human serum albumin (HSA) and fibrinogen (HFB) adhere to the copolymer surface and to which extent binding is irreversible. 0.5% copolymer solutions and 0.05 % (weight/volume) protein solutions were used in this study. Copolymer solutions were spin-coated onto silver coated glass plates and the shifts in resonance angle ($\Delta\theta_{spr}$) expressed in millidegrees of angle (mdA) obtained for both HSA and HFB, as measured by a surface plasmon resonance instrument (WBI006, Johnson & Johnson Clinical Diagnostics Ltd., England) are provided in the following table 2.

Table 2

Copolymer of example No	$\Delta\theta_{spr}$ (HAS)	$\Delta\theta_{spr}$ (HFB)
3	65	438
4	61	394
5	115	526
6	115	594
7	114	529
9 (PEG 400)	120	439
9 (PEG 4,000)	99	411
13 B	82	345
13 E	65	242
14	134	469

The steep slopes of the SPR curves prove the fast adsorption of proteins, a maximal adhesion being observed already after 40 seconds. Upon overflowing a second time, no extra adhesion was observed, indicating the formation of a protein monolayer. All copolymers, except for that of example 13, adhered a monolayer of fibrinogen. Important variations of $\Delta\theta_{spr}$ (HFB) within a range of about 350 to 600 mdA are believed to be due to the diiferent ways according to which fibrinogen can adhere to the copolymer surface: in particuler, the high values of $\Delta\theta_{spr}$ (HFB) for the copolymers of example 6 and 7 are deemed to be due to the presence of a positively charged nitrogen group strongly interacting with the negatively charged fibrinogen (pI = 5.5) at physiological conditions (pH = 7.4).

EXAMPLE 16 – protein adhesion and substitution to a copolymer surface measured through surface plasmon resonance.

The copolymer of example 3 was tested as follows for protein adsorption and substitution upon subsequent contact with single component solutions of albumin, fibrinogen and immunoglobulin G (available from Sigma Immunochemicals).

In a first experiment the $\Delta\theta_{spr}$ for maximal amounts of adhered protein were determined. Next, the protein layer was reacted with its corresponding monoclonal antibody. The second shift in resonance angle, due to the binding of the antibody, is also a measure for the amount of protein adhered.

Substitution experiments were then performed, in which the surface composition, protein concentration and flow speed were kept constant. From a 0.05% weight/volume HSA solution, in the first channel a maximal amount of HSA adhered and was quantified via binding with the HSA-antibody. The shift in resonance angle ($\Delta\theta_{spr1}$) was related to maximal HSA-binding (100% HSA present). Next, in the second channel, after an albumin solution, a fibrinogen solution was pumped over the surface. The second angle shift ($\Delta\theta_{spr2}$) was due to the substitution of albumin by fibrinogen molecules. Using the HSA-antibody, the percentage HSA still present in the adhered protein layer was determined by dividing the antibody response by the antibody response for maximal HSA-adsorption. In the third channel, the HSA solution was followed by a human immunoglobulin-G (HigG) solution. The sensorgram of the various HSA-antibody responses is provided in figure 2 and the data from the substitution experiments ($\Delta\theta_{spr}$ being expressed in mdA) are presented in the following table 3.

Table 3

Protein layer	$\Delta\theta_{spr1}$ (mdA)	$\Delta\theta_{spr2}$ (mdA)	HSA antibody response	%HSA present
HAS	66		234	100
HSA/HFB	64	58	177	76
HSA/HigG	69	217	68	29
HFB	314		0	0

Table 3 (following)

Protein layer	$\Delta\theta_{spr1}$ (mdA)	$\Delta\theta_{spr2}$ (mdA)	HFB antibody response	%HFB present
HFB	322		113	100
HFB/HAS	320	- 43	131	100
HFB/HigG	344	104	99	88
HAS	314		0	0

Protein layer	$\Delta\theta_{spr1}$ (mdA)	$\Delta\theta_{spr2}$ (mdA)	HigG antibody response	%IgG present
HigG	378		344	100
HIgG/HSA	367	- 5	344	100
HigG/HFB	352	88	224	65
HAS	72		41	12

From the above results, it may be concluded that HFB and HIgG possess a high affinity for the hydrophobic fluorinated copolymer surface and that they replace HSA reversibly. Upon substitution, an equilibrium with both HFB and HIgG present at the surface is obtained. The substituted protein fraction depends on the extent to which the first adhered protein is able to relax and bind irreversibly.

EXAMPLE 17 – competitive adsorption of plasma proteins to a copolymer surface measured through surface plasmon resonance.

The competitive adsorption of albumin, fibrinogen and immunoglobulin G from diluted plasma to the surface of the copolymer of example 3 was followed via SPR versus time. Diluted human plasma was pumped over the copolymer surface at various time intervals (5 s to 500 s) with a flow speed of 2 µl/s. For each time interval, the concentration of each protein was determined via binding to the corresponding antibody. In order to obtain the percentage of protein coverage after a certain time period, $\Delta\theta_{spr}$ was divided by the antibody response for maximal

protein adsorption. Results are provided in the following table 4 and graphically shown in figure 3.

Table 4

Time (seconds)	HSA response mdA	HSA response %	HFB response MdA	HFB response %	HigG response MdA	HIgG response %
Maximal Adsorpt.	234	100	113	100	344	100
5	161	68	46	41	107	31
15	140	60	39	35	116	33
30	132	56	32	28	147	43
60	120	51	33	29	157	45
100	127	54	27	24	172	50
200	98	41	25	22	188	55
300	76	32	18	16	185	54
400	71	30	20	18	145	42
500	80	34	20	18	140	40

From the above results, it can be seen that no Vroman effect was observed. The proteins adhered very fast to the surface. From the SPR-sensogram it can be deduced that within the first 100 seconds a stable plasma protein layer is built and substitution of proteins is still possible.

EXAMPLE 18 – Effect of irradiation sterilization on the macromolecular characteristics of terpolymers.

Terpolymers of the previous examples were submitted to gamma sterilization via ^{60}Co at various irradiation doses. Number average molecular weights M_n and polydispersity index d (defined as M_w/M_n) of the copolymers were determined by gel permeation chromatography as indicated hereinbefore and are provided in table 5 below.

Table 5

Copolymer of example No.	0 kGy M_n	0 kGy d	10 kGy M_n	10 kGy d
3	89,000	1.7	88,200	1.9
4	123,000	1.8	126,000	1.8
5	40,000	3.0	39,400	3.0
9 (PEG 400)	40,600	2.4	44,600	3.2
9 (PEG 4,000)	80,800	2.0	100,000	3.0
13 B	29,400	1.9	31,600	2.1
13 C	55,500	1.8	54,500	2.3
13 E	50,100	1.5	54,000	1.7
14	46,600	2.4	49,000	2.8

Copolymer of example No.	25 kGy M_n	25 kGy d	50 kGy M_n	50 kGy d
3	91,300	2.0	87,600	2.4
4	122,000	2.0	116,400	2.2
5	43,100	3.8	52,100	4.3
9 (PEG 400)	49,500	3.7	39,900	4.7
9 (PEG 4,000)	93.600	3.3	61,500	5.5
13 B	31,200	2.3	34,500	2.7
13 C	69,500	2.1	54,100	3.6
13 E	50,100	1.9	48,200	2.6
14	53,800	2.7	55,600	3.2

From the above data it can be concluded that although some increase in the molecular weight and some broadening of the molecular weight range occurs with increasing irradiation doses, these modifications in the macromolecular structure

are not susceptible to significantly alter the mechanical properties, in particular the elasticity of these terpolymers as required in biomedical applications.

EXAMPLE 19 – *in vitro* endothelization of an ionic terpolymer.

Microscopy glasses (2.7 x 7.6 cm x 0.1 cm) were silanated and dipcoated with a 2 % weight/volume solution of the ionic terpolymer of example 7. After ethylene oxide sterilization, their surfaces were seeded with human umbelial vein endothelial cells (HUVEC) at a concentration of 36,000 cells/cm^2. After 24 hours incubation, the coated glasses were placed under sterile conditions into a flow chamber where they were brought into contact with a phosphate buffer saline (PBS) flow. Culture medium was pumped over the surface, first under a venous pressure (0.1 Pa, 10 minutes) and later under arterial pressure (0.74 Pa, 20 minutes). Using a phase contrast video camera focused and fixed on a selected region in the middle of the chamber, a picture of the endothelial cell layer was recorded each 5 minutes (magnification:200 x). After 30 minutes of flow, the chamber was rinsed with phosphate buffered saline. The set of pictures proved that the ionic terpolymer adheres well to endothelial cells under static conditions. This observation is in contrast with the teachings of the literature, namely P.Van Wachem in *Biomaterials* (1985) 6:403, reporting a poor cell adhesion to hydrophobic fluorinated surfaces.

EXAMPLE 20 – study of *in vitro* bacterial adhesion via radiolabelling.

The presence of *Staphylococcus epidermis*, the most frequent pathogen of infested implants, was studied via radiolabelling (e.g. ^{3}H-adenin). The "bacterial adhesion to hydrocarbons" (BATH) test was used for this purpose since it is widely recognized for its accuracy to test the relative hydrophobicity of micro-organisms, based on the adhesion to n-decane.

For quantification of the adhered micro-organisms by means of radio labelling, the samples were prepared by coating silanated scintillation bottles with a 2% (weight/volume) copolymer solution. As reference materials, polymer plates (2.5 x 3 cm) made of polyurethane (Chronoflex-80A, Cardio.Tech. Int., Italy), low density polyethylene LDPE (Quantum Chemical Corporation, USA) and polystyrene (PS)

were prepared by means of a mechanical press (10 kN) at 200 °C. Glass and stainless steel (SS, Inox 64 K°, Metaplast) plates were also used as reference materials.

Staphylococcus epidermis (LMG 10474, ATCC 11775) was obtained from the Culture Collection of the University of Gent and cultured on a nutrient agar (pH 7.4) supplemented with 1% glucose. Growth was followed by means of absorbancy measurements at 550 nm (Vitalab 10 photometer, Vital Scientific). Only *Staphylococcus epidermis* in the stationary growth phase (after more than 10 hours of incubation) was used for the experiments. The hydrophobicity of the bacterial surface was determined following the method of Rosenberg. Several volumes of n-octane (0.1 –1 ml) were added to 3 ml of the bacterial suspension in phosphate buffer (9.5 x 10^8 cells). After two minutes shaking, two separated phases were obtained again after 15 minutes, then the aqueous phase was removed. The difference in absorbancy between the original and the final suspension is a measure for the bacterial hydrophobicity.

Staphylococcus epidermis was radioactively labelled with (2,8-^{3}H)-adenine (Dupont, Belgium, specific activity 288,000 Ci/mol, conc. 10,000 Ci/ml). 100 µl (100 µCi) of ^{3}H-adenine was carefully smeared with a sterile trihalsky over a nutrient agar plate recently inoculated with *Staphylococcus epidermis*. After 16 hours of incubation at 37°C, the micro-organisms were harvested (1M KH_2PO_4, Na_2HPO_4, phosphate buffer pH= 7.4, 15ml/plate) and concentrated via centrifugation (Serval Superspeed RC-2, 2500 g, 10 min). The bacterial pellet was slowly brought into suspension again with the phosphate buffer, in order to reach a final concentration of 1.8 x 10^9 cells/ml (Abs= 1.5). The activity of this suspension was measured and the specific activity determined (2.7 x 10^{-4} counts/micro-organism). Next, the radioactive suspension was brought during one hour into contact with the polymer coatings and the reference plates. The scintillation bottles were mounted in a rotating disc and rotated at 60 rpm through a water bath at 37°C. A dynamic bacterial flow was secured and the contact with the materials was favoured. The materials were washed twice with phosphate buffer in order to remove reversibly

adhered bacteria (3 minutes at 60 rpm). After 3 minutes sonication of the materials in contact with a third volume of phosphate buffer, a sample (20 µl) was taken and scintillation liquid (10 ml, Ultima Gold LSC Cocktail, Hewlett Packard) added. The radioactivity was measured via a liquid scintillation spectrometer (Beckman LS-100, Analis, 20 minutes). Results are shown on figure 4 for the copolymers and terpolymers of examples 3, 4, 7, 9 (PEG 4,000), 13 E and 14. These data demonstrate that the strongly fluorinated copolymer of example 4 and, to a lower extent, the ionic terpolymer of example 14, adhere extremely high concentrations of *Staphylococcus epidermis*. On the other hand, the copolymer of example 3 and the polyethyleneglycol-containing terpolymers of examples 9 and 13 E exhibit the lowest adhesion.and therefore are promising materials to reduce bacterial adhesion.

EXAMPLE 21 – determination of the bacterial resistance of a copolymer via adenosine triphosphate (ATP) intracellular concentration.

For quantification of the adhered micro-organisms by means of ATP determination, the samples were prepared by coating Eppendorf tubes with a 2% (weight/volume) copolymer solution. Polypropylene (PP) was used as the reference material. The micro-organism (*Staphylococcus epidermis*) was selected and prepared in the same way as in example 20. The ATP-kit (Bio-orbit Oy, Finland) is based on the ATP-dependent behaviour of luciferase. *Staphylococcus epidermis* was cultured on nutrient agar plates overnight at 37°C, harvested, centrifuged (Sorvall RT 6000B, 20 min, 3500 rpm) and washed twice. The pellet was resuspended in phosphate buffer until a dense suspension of 1.8×10^9 cells/ml was obtained (Spectrophotometer Pharmacia LKB, Novaspec II, 550 nm). The coated Eppendorf tubes were brought into contact with the bacterial suspension for 1 hour at 37°C (Innova incubator 4230, 150 rpm) then washed five times by adding 1 ml of phosphate buffer and suctioning this again. The adhering micro-organisms were lysed by 5 to 10 minutes incubation with 1 ml of a trichloroacetic acid (TCA) solution (TCA 1%, EDTA 2mM, xylenol blue 0.002% in distilled water). A 20 µl sample was pipetted in a 96-well microtiter plate (Dynatech

Microlite 1). The pink lytic solution was neutralized with 180 µl tris acetate buffer, as a consequence of which a change in colour (pink to yellow) took place. The microtiter plate was kept on ice. Measurements were effected using an Amerlite illuminometer (Amersham) at a maximal emission wavelength of 562 nm. First the background noise of 40 µl of luciferase was measured (background value), next 150 µl of the bacterial sample was pipetted over and the emitted signal registered once more (sample value). Finally, 50 µl of an internal ATP- standard solution was added to each well (standard value). The ATP-level per sample was calculated as follows: [(sample value – background value)/(standard value – sample value)]x µ mole added standard. Results are shown on figure 5 for the copolymers and terpolymers of examples 3, 4, 7, 9 (PEG 4,000), 13 E and 14. These data confirm the results obtained by the adenosine triphosphate concentration method of example 21, i.e. the copolymer of example 3 and the polyethyleneglycol-containing terpolymers of examples 9 and 13 E are amenable to reduce bacterial adhesion.

EXAMPLE 22 - *in vivo* encrustation resistance of a poly(octafluoropentyl-methacrylate-co-2-ethylhexylacrylate)

Eight discs (3 mm diameter, 1 mm thick) of the copolymer of example 3 were punched out of a pressed polymer plate. Discs made from materials used in commercial catheters like silicon (Bard, urinary catheter) and polyurethane (Chronoflex, Cardio.Tech.Int., Italy) were used as reference materials. After X-ray sterilization, the discs were implanted into the bladder of adult male Wistar rats (± 200 g) under sterile conditions, full narcosis (2 to 3% halothane in oxygen) and administration of a single dose of antibiotics (Clamoxil®, 15 mg/kg). An incision through the subcutanic tissue and the peritoneum led to the bladder. Urine pH was measured and uropathogenic micro-organisms determined. A polymer disc was sutured with vicryl suture thread into the mucus wall, in order to impair obstruction of the passage to the urether. After suture, the wound was rinsed thoroughly with an isobetadin solution. A long-acting analgesic (brupenorphin, 0.3 mg/kg) was administered subcutanously. After 9 weeks, the rats were killed via an overdose CO_2. Again, urine pH was determined and microbiological analyses performed.

The polymeric discs were collected and the deposited calcium salts dissolved with 10 ml of an acidic lanthanum chloride solution. The amounts of calcium were determined via atomic absorption spectroscopy for each type of disc and expressed in μ mole. Results were as follows:

2.51 μ mole for the copolymer of example 3

3.17 μ mole for silicon

1.83 μ mole for polyurethane

These data indicate that the copolymer of example 3 provides a low encrustation value under the conditions of this experiment. This finding, combined with its low bacterial adhesiveness demonstrated in examples 20 and 21, makes this polymer a good candidate for coating urological catheters and stents.

EXAMPLE 23 – drug-loaded copolymer coatings obtained by dip-coating.

Methylprednisolon (MP) (Sigma Chemicals) and valsartan (VAL) (Ciba Geigy AG, Basel, Switzerland) were incorporated into a coating of the copolymer of example 3. Using the dip coating technique, stainless steal plates (2x1x0.1 cm^3) were immersed trice in a 2% (weight/volume) solution of drug and polymer, a $CHCl_3$/acetone (85/15) mixture being used as the solvent. Respectively 5 and 10% by weight drug relative to the copolymer was incorporated. Scanning electron microscopy by means of an SEM 505 apparatus (Philips, 30 kV, WDX-24 electron accelerator) showed the presence of a 225 nm thick copolymer layer with a predominance of fluorinated units at its surface. The inclusion of drugs did not result in topographic changes.

EXAMPLE 24 – drug-loaded copolymer coatings obtained by spray-coating.

Solutions (100 mg/10 ml) of the copolymer of example 3, in which respectively 9, 33 and 50% by weight of a drug (MP or VAL) was dissolved or suspended, were used for the spray coating of stents. The stents were mounted on a rotary platform and spray-dusted via capillary atomisation under air pressure. A barrier coating was applied by, after 2 hours drying of the first polymer layer, atomizing for a second time 10 ml of the 1% (weight/volume) solution of the copolymer over the stents. Scanning electron microscopy by means of an SEM

505 apparatus showed the presence of a 5 µm thick copolymer layer. A rough topography and/or drug spots were observed at the surface for coatings loaded with 50% by weight of a drug. 9 % by weight loded spay coatings had a flat surface.

EXAMPLE 25 – *in vivo* biocompatibility of polymer coated stents

Balloon expandable, stainless steal stents, 16 mm long and with specific zigzag shape were folded out of a 0.18 mm diameter thread. The stents were provided with a coating of the copolymer of example 3 by dipping or spraying according to the methods of examples 23 and 24. Dust-free drying was effected at room temperature. The stents were sterilized by X-ray irradiation at 25kGy.

Cross-bred pigs (20-25 kg) were used as laboratory animals, a standard grain diet without any fat or cholesterol supplements being fed to them. The animals were treated according to the standards of the 'National Institute of Health Guide for the care and use of laboratory animals'. Under full narcosis, the stents were implanted in the crown/coronary artery of the animals. Heparin and acetylsalicylic acid were administered during the operation. Via the thigh artery, per pig, one (coated or non-coated) stent with a conventional 3.0 mm coronary angioplastic balloon catheter was placed in the right coronary and expanded under a pressure of 8 atm. during 60 seconds. By means of coronary angiography the procedure was being followed. During the follow-up, anti-blood platelet agents or anticoagulants were no longer administered. After 6 weeks, a control angiography was performed on the stented coronary artery. An angiographic analysis of the lumen diameter, before, just after and 6 weeks after implantation, was performed with a Polytron 1000®-apparatus (Siemens AG, Germany). Iohexol (Omnipaque, Nycomed, Oslo) was used as contrast fluid. The calibration wad one by means of a metal bar. The pigs were killed by intravenous administering of 10 ml of a saturated NaCl solution. The right coronary was fixed under pressure (80 mm Hg) with a 10% formalin solution. The stented segment (from 1 cm proximal to 1 cm distal from the stent) was carefully dissected and fixed in a 2% formalin solution. The stent thread was removed with a stereomicroscope, avoiding thereby deformation of or damage

to the artery. The segments were imbedded in a cold-polymerising holder (Technovit 710-Heraeus Kulzer GmbH, Germany) and 5 µm thick sections were prepared with a microtome (HM 360, Microm, Germany). The microscopic sections were stained with haemalaun-eosin, Masson's trichome, elastica von Gieson and phophotungstic acid heamatoxylin (PTAH). The tissue sections/samples were studied with a light microscope by an experienced pathologist, who was unaware of the type of coating. Morphometric analyses were performed via a computer steered program (Leitz CBA 8000). For the statistical processing of the angiographic measurements, a paired t-test was used. For comparison of two different groups (coated-not coated, with-without drug), the non-paired t-test was used. The data were presented as mean values ± standard deviations. Measurements with a p-value below 0.05 were considered to differ significantly.

CLAIMS

1. A copolymer comprising moieties derived from at least one first fluorinated comonomer having the formula :

$$CH_2 = \underset{\displaystyle X}{\overset{\displaystyle |}{C}} - COOCH_2\, Y(CF_2)_n\, CF_2Z \qquad (I)$$

wherein

- X is hydrogen or methyl,
- Y is a single bond or a radical of the formula CH_2NRSO_2 or $NRSO_2$, R being a $C_{1\text{-}6}$ alkyl group,
- Z is hydrogen or fluorine, and
- n is 0 to 12

and moieties derived from at least one second non-fluorinated comonomer, the glass transition temperature of the homopolymer of said second comonomer being lower than the glass transition temperature of the homopolymer of said first comonomer.

2. A copolymer according to claim 1, wherein the first comonomer is selected from trifluoroethyl methacrylate, octafluoropentyl methacrylate, dodecafluoroheptyl methacrylate, pentadecafluorooctyl methacrylate, heptadecafluorooctyl ethylsulfonamidoethyl acrylate and heptadecafluorooctyl butylsulfonamidoethyl acrylate.

3. A copolymer according to claim 1 or claim 2, wherein the second comonomer is an acrylic monomer.

4. A copolymer according to any of claims 1 to 3, wherein the glass transition temperature of the homopolymer of said second comonomer is not above 5°C.

5. A copolymer according to any of claims 1 to 4, wherein the second comonomer is 2-ethylhexyl acrylate.

6. A copolymer according to any of claims 1 to 5, having a multi-sequence structure with variable proportions of the first comonomer and of the second comonomer in each sequence.

7. A copolymer according to any of claims 1 to 6, comprising at least one block derived from the homopolymer of the first comonomer and/or at least one block derived from the homopolymer of the second comonomer.

8. A copolymer according to any of claims 1 to 7, further comprising moieties derived from at least one hydrophilic third comonomer copolymerizable with the first comonomer and the second comonomer.

9. A copolymer according to claim 8, wherein the said hydrophilic third comonomer is an acrylic monomer.

10. A copolymer according to claim 8, wherein the said hydrophilic third comonomer is selected from methacrylic acid, N,N-dialkylaminoalkyl methacrylates, quaternized N,N-dialkylaminoalkyl methacrylates and polyalkyleneglycol-containing monomers.

11. A copolymer according to claim 8, wherein at least one said hydrophilic third comonomer is methacrylic acid and wherein the said copolymer is in the form of an aqueous solution.

12. A copolymer according to any of claims 1 to 11, further comprising moieties derived from at least one non-hydrophilic fourth comonomer copolymerizable with the first comonomer and the second comonomer.

13. A copolymer according to claim 12, wherein the said non-hydrophilic fourth comonomer bears at least one functional group which is able to react with amino groups.

14. A copolymer according to claim 12 or claim 13, wherein the said non-hydrophilic fourth comonomer is succinimidyl methacrylate.

15. A copolymer according to any of claims 1 to 14, having a glass transition temperature in the range of -20°C to 20°C.

16. A copolymer according to any of claims 1 to 15, having a number average molecular weight in the range of 25,000 to 200,000.

17. A copolymer according to any of claims 1 to 16, having a molecular weight polydispersity in the range of 1.3 to 5.5.

18. A copolymer according to any of claims 1 to 17, in the form of a layer having a thickness in the range of 0.1 to 15 µm.

19. A layer of a copolymer according to any of claims 1 to 17, further comprising a biologically effective amount of at least one biologically active ingredient.

20. A biomedical device coated with at least one layer of a copolymer according to any of claims 1 to 18 or with at least one layer according to claim 19.

21. A biomedical device according to claim 20, in the form of an implant.

22. A biomedical device according to claim 20, in the form of a catheter.

23. A biomedical device according to claim 20, in the form of a stent comprising a stent body having a metal surface and wherein the copolymer layer is coated onto at least a part of the said metal surface.

24. Use of a copolymer according to any of claims 1 to 17 as a substrate for seeding of endothelial cells.

25. A method for treating atherosclerosis by making use of a coronary stent coated with at least one layer of a copolymer according to any of claims 1 to 18 or with at least one layer according to claim 19.

26. A process for making a copolymer according to any of claims 1 to 18, comprising providing a mixture comprising :

- at least one first fluorinated comonomer having the formula (I),
- at least one second non-fluorinated comonomer, the glass transition temperature of the homopolymer of said second comonomer being lower than the glass transition temperature of the homopolymer of said first comonomer,
- optionally at least one hydrophilic third comonomer copolymerizable with the first comonomer and the second comonomer,
- optionally at least one non-hydrophilic fourth comonomer copolymerizable with the first comonomer and the second comonomer,
- at least one solvent for the said first fluorinated comonomer and the said second non-fluorinated comonomer, and
- at least one free radical initiator

to a reactor and submitting the said reactor to temperature and pressure conditions under which the said first fluorinated comonomer and the said second non-fluorinated comonomer are able to copolymerize, and optionally further removing the said solvent.

27. A process according to claim 26, wherein the said solvent is selected from 1,1,1-trichloroethane, trichlorotoluene, trifluorotoluene and their mixtures.

28. A process according to claim 26 or claim 27, wherein the said free radical initiator is selected from azoic compounds, polyalkyleneglycol-containing macro-initiators and peroxides.

29. A process according to any of claims 26 to 28, wherein the said temperature conditions include a temperature not exceeding the boiling point of the solvent.

30. A process for making a coated stent, comprising coating a stent body having a metal surface with at least one polymer, wherein the said comprises a copolymer according to any of claims 1 to 18 or a copolymer layer according to claim 19.

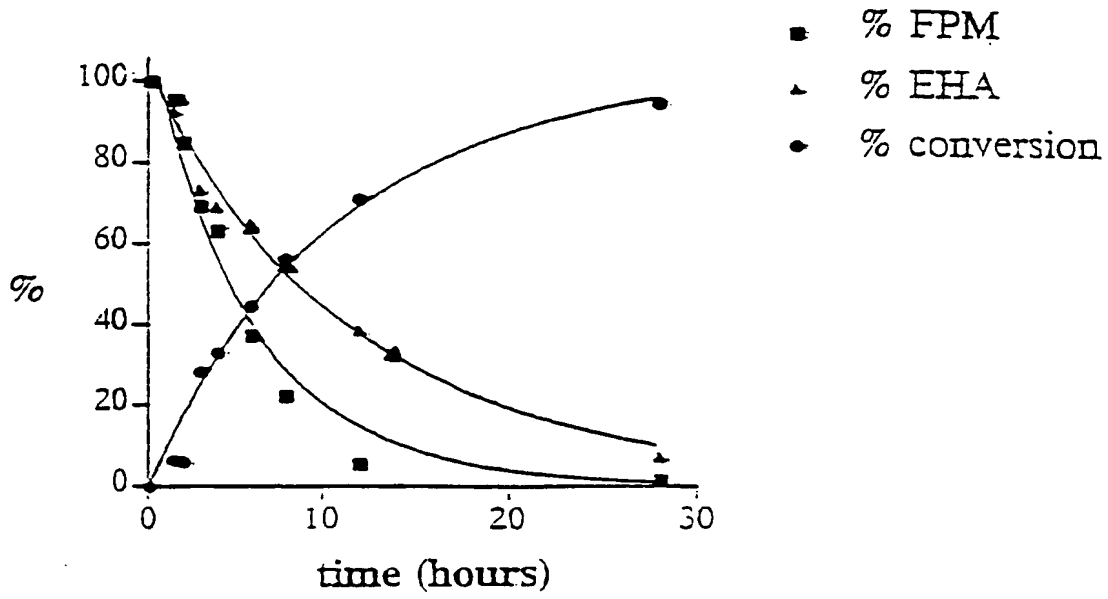


Fig. 1

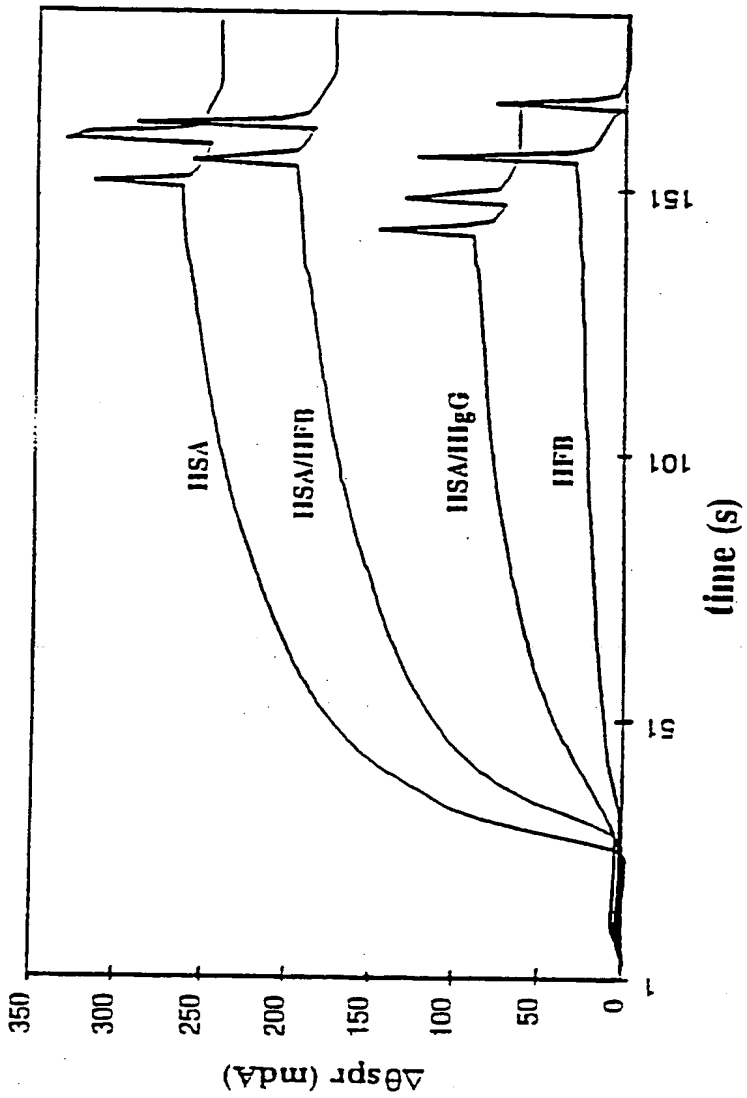
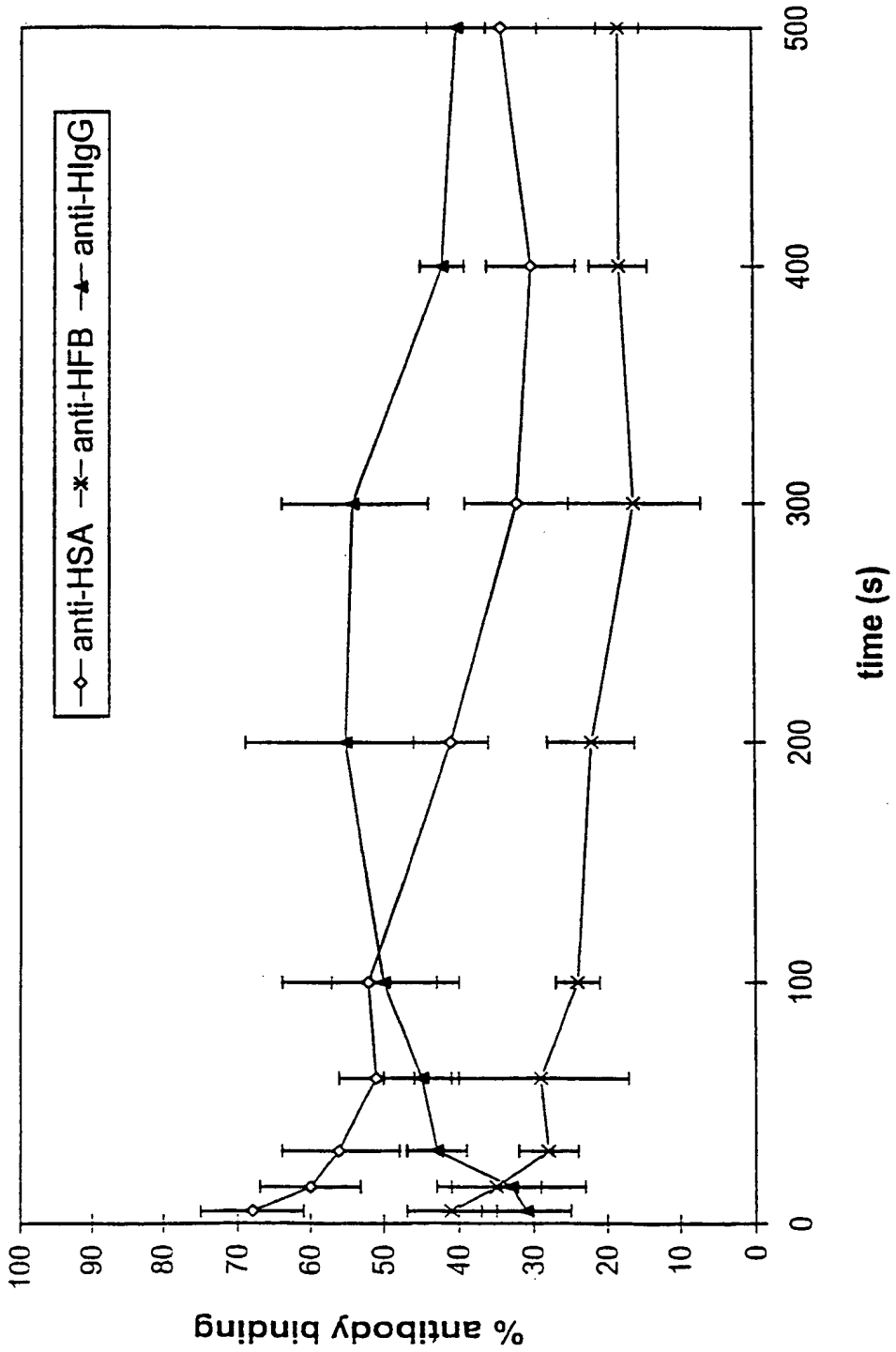


Fig. 2

Fig.3


anti-HSA
anti-HFB
anti-HIgG
% antibody binding
time (s)
0
10
20
30
40
50
60
70
80
90
100
0
100
200
300
400
500

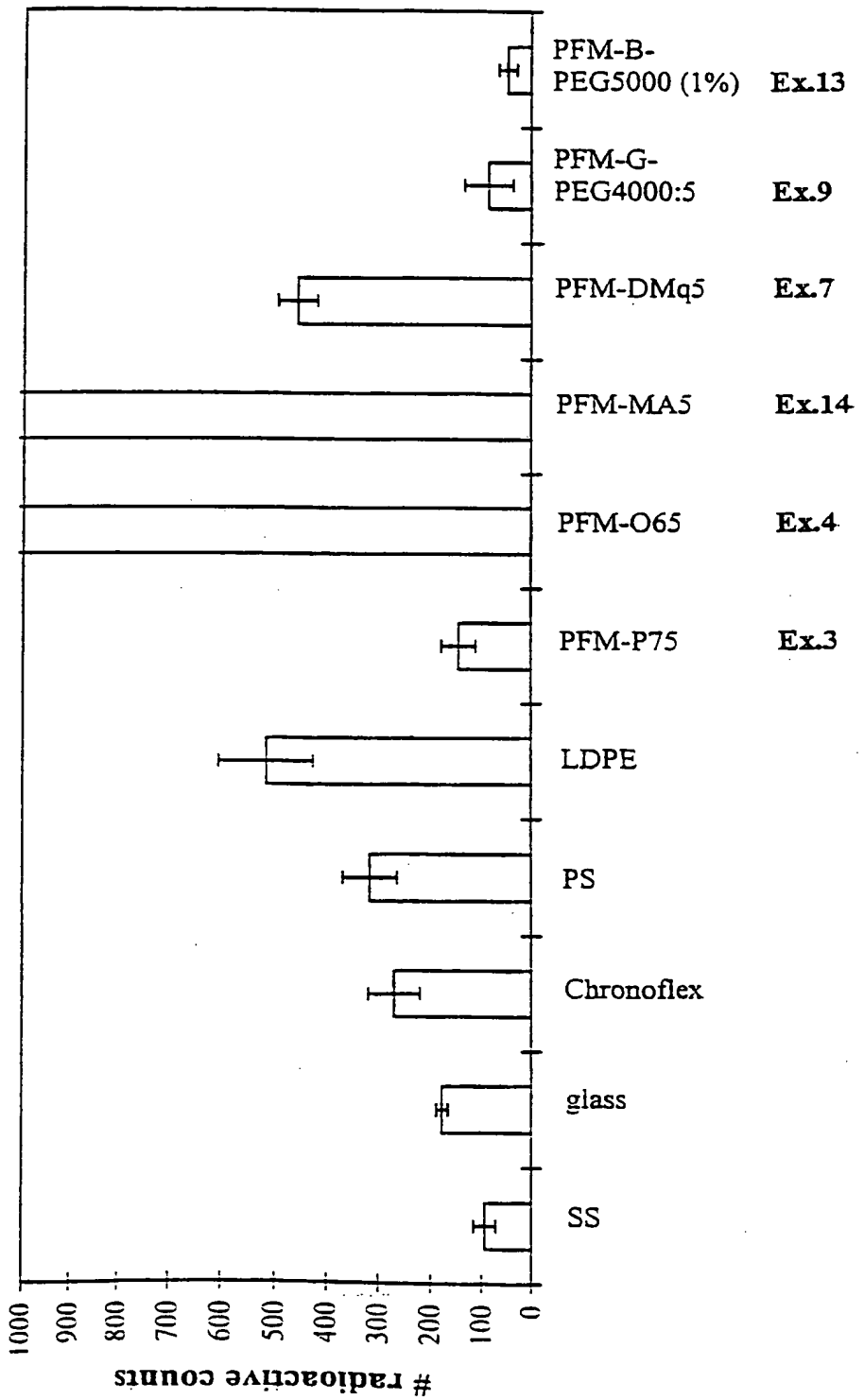


Fig.4

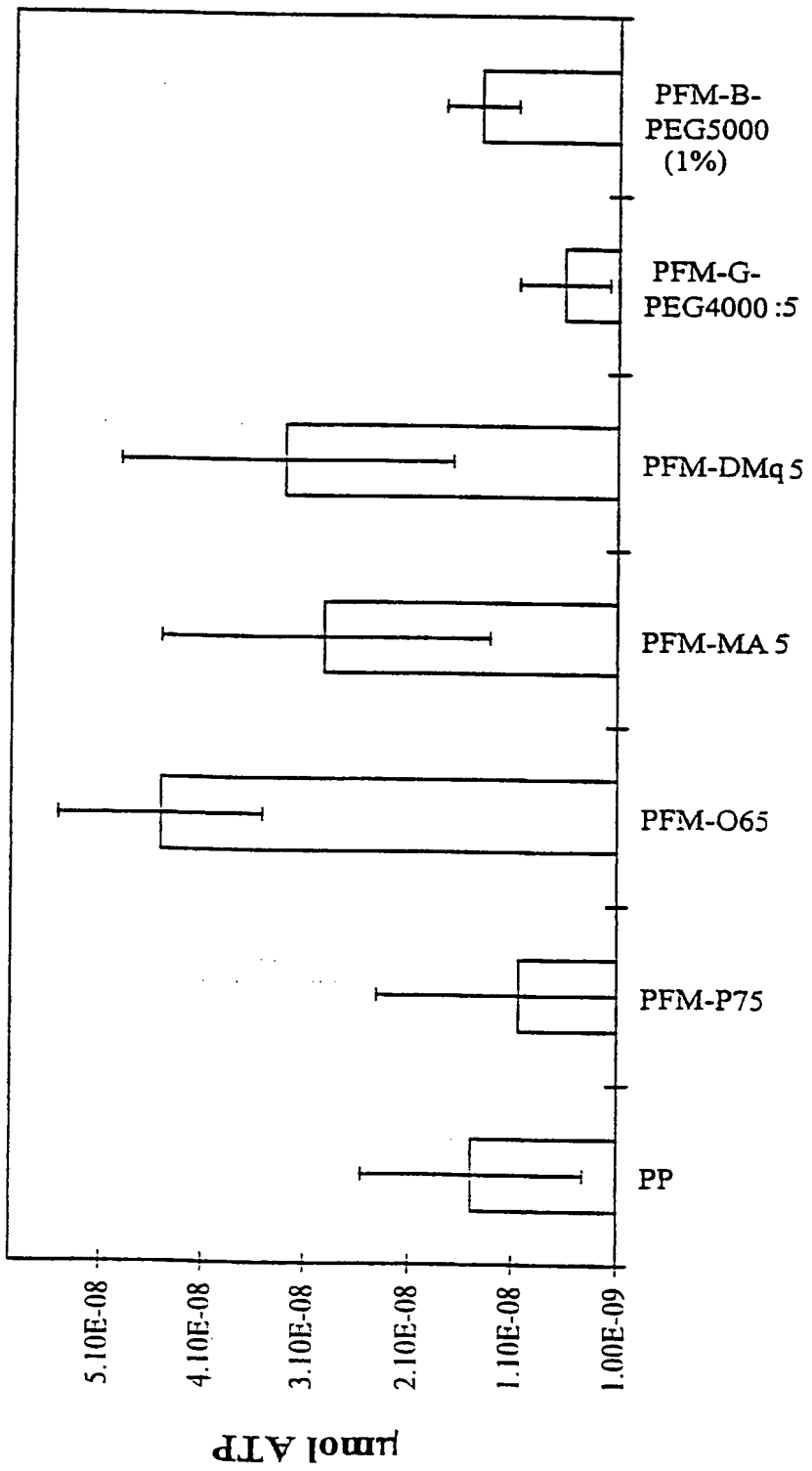


Fig.5

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/EP 00/02733

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08F220/22 C08F297/02 A61L31/10 A61L29/08 A61L27/34
A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L C08F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 489 560 A (HITACHI CHEMICAL CO LTD) 10 June 1992 (1992-06-10) claims 1,2 page 3, line 28,42,43 page 4, line 26,27,30,47,51-53 page 5, line 1-4 page 10, line 35 ---	1-5, 8-10,12, 15,16, 18,26,28
X	US 5 798 406 A (FERET BRUNO ET AL) 25 August 1998 (1998-08-25) examples 1-16 claims 1,4,7,8,17,19,23,25,31,32 --- -/--	1-10,12, 15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2000	08/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rose, E

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INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/EP 00/02733

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	EP 0 506 067 A (UNION CARBIDE CHEM PLASTIC) 30 September 1992 (1992-09-30) page 8, line 34 -----	1-30

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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